

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claim 1 (Previously Presented): A bioreactor comprising:

a housing defining a first chamber that contains a first liquid medium, the housing including an inlet port and an outlet port for fluid flow of the liquid medium through the first chamber, the liquid medium including at least one of a growth or culture medium for growing or culturing cells in the first chamber;

at least one gas permeable membrane defining at least a portion of the housing, the membrane allowing gas flow through the housing into the first chamber; and

a hydrostatic loading module that transmits hydrostatic pressure through the membrane to the first liquid medium contained in the first chamber.

Claim 2 (Original): The bioreactor of claim 1, the hydrostatic loading module transmitting the pressure by a static second liquid medium.

Claim 3 (Original): The bioreactor of claim 1, the hydrostatic loading module being attached to the housing and forming a second chamber with the housing, the second chamber containing a second liquid medium and being separated from the first chamber by the gas permeable membrane.

Claim 4 (Original): The bioreactor of claim 3, the hydrostatic loading module including at least one pump for increasing or decreasing the pressure of the second liquid medium in the second chamber.

Claim 5 (Original): The bioreactor of claim 3, the hydrostatic loading module being capable of increasing or decreasing the hydrostatic pressure in the first chamber.

Claim 6 (Original): The bioreactor of claim 4, the hydrostatic loading module further including a pressure sensor for monitoring the pressure in the second chamber.

Claim 7 (Original): The bioreactor of claim 1, the housing including a frame, the frame including a first surface, a second surface spaced apart and aligned with the first surface, and an opening that extends through the frame from the first surface to the second surface.

Claim 8 (Original): The bioreactor of claim 7, the housing including a first gas permeable membrane attached to the first surface of the frame and a second gas permeable membrane attached to the second surface of the frame, the first gas permeable membrane, the second gas permeable membrane, and the frame defining the first chamber.

Claim 9 (Original): The bioreactor of claim 1, the hydrostatic loading module being attached to the housing and including a second chamber and a third chamber, the second chamber and the third chamber containing a second liquid medium and being separated from the first chamber by, respectively, a first gas permeable membrane and a second gas permeable membrane.

Claim 10 (Original): The bioreactor of claim 1, the at least one gas permeable membrane having sufficient optical transparency to permit visual observation of the first chamber.

Claim 11 (Previously Presented): The bioreactor of claim 10, the at least one gas permeable membrane being resistant to cell attachment.

Claim 12 (Original): The bioreactor of claim 1, further including a pH sensor, the pH sensor measuring the pH of the first liquid medium entering the first chamber and exiting the first chamber.

Claim 13 (Original): The bioreactor of claim 1, further including an impeller for circulating the first liquid medium in the first chamber.

Claim 14 (Currently Amended): The bioreactor of claim 1, ~~the inlet port~~ further including a first flow control valve positioned in the inlet port and the outlet port ~~including a second flow control valve~~ positioned in the outlet port, the first flow

control valve and the second flow control valve regulating the flow of the first liquid medium through the first chamber.

Claim 15 (Withdrawn): A bioreactor comprising:

a housing defining a first chamber, a second chamber, and a first gas permeable membrane separating the first chamber and the second chamber and allowing gas flow between the first chamber and the second chamber, the first chamber containing a first liquid medium and including an inlet port and an outlet port for fluid flow of the first liquid medium through the chamber, the first liquid medium being used to culture or grow cells or tissue in the first chamber, the second chamber containing a second liquid medium and including an inlet and outlet for fluid flow of the second liquid medium through the second chamber; the hydrostatic pressure of the second liquid medium being transmitted through the first gas permeable membrane to affect the hydrostatic pressure of the first liquid medium contained in the first chamber.

Claim 16 (Withdrawn): The bioreactor of claim 15, further including at least one pump for increasing or decreasing the pressure of the second liquid medium in the second chamber.

Claim 17 (Withdrawn): The bioreactor of claim 16, further including a pressure sensor for monitoring the pressure in the second chamber.

Claim 18 (Withdrawn): The bioreactor of claim 15, the housing including a frame, the frame including a first surface, a second surface spaced apart and aligned with the first surface, and an opening that extends through the frame from the first surface to the second surface.

Claim 19 (Withdrawn): The bioreactor of claim 18, the gas permeable membrane being attached to the first surface of the frame, and the housing further including a second gas permeable membrane attached to the second surface of the frame, the first gas permeable membrane, the second gas permeable membrane, and frame defining the first chamber.

Claim 20 (Withdrawn): The bioreactor of claim 19, including a third chamber, the third chamber containing the second liquid medium and being separated from the first chamber by the second gas permeable membrane.

Claim 21 (Withdrawn): The bioreactor of claim 20, the first gas permeable membrane and the second gas permeable membrane having sufficient optical transparency to permit visual observation of the first chamber.

Claim 22 (Withdrawn): The bioreactor of claim 15, the first gas permeable membrane being resistant cell attachment.

Claim 23 (Withdrawn): The bioreactor of claim 15, further including a pH sensor, the pH sensor measuring the pH of the first liquid medium entering the first chamber and exiting the first chamber.

Claim 24 (Withdrawn): The bioreactor of claim 15, further including an impeller for circulating the first liquid medium in the first chamber.

Claim 25 (Withdrawn): The bioreactor of claim 1, the inlet port including a first flow control valve and the outlet port including a second flow control valve, the first flow control valve and the second flow control valve regulating the flow of the first liquid medium through the first chamber.

Claim 26 (Withdrawn): A bioreactor comprising:

- a housing defining a first chamber that contains a first liquid medium and a plurality of cells, the housing including an inlet port and an outlet port for fluid flow of the liquid medium through the first chamber, the liquid medium including at least one of a growth or culture medium for growing or culturing the plurality of cells in the first chamber;
- at least one gas permeable membrane defining at least a portion of the housing, the membrane allowing gas flow through the housing into the first chamber;
- and

a hydrostatic loading module for transmitting hydrostatic pressure through the membrane to the first liquid medium and the plurality of cells contained in the first chamber.

Claim 27 (Withdrawn): The bioreactor of claim 26, the plurality of cells contained in the first chamber being seeded on at least one of a scaffold or sponge.

Claim 28 (Withdrawn): The bioreactor of claim 27, the plurality of cells comprising mesenchymal stem cells.

Claim 29 (Withdrawn): The bioreactor of claim 28, the mesenchymal stem cells being treated with a cytokine to promote differentiation into chondrogenic tissue.

Claim 30 (Withdrawn): The bioreactor of claim 28, the mesenchymal stem cells being aggregated prior to being seeded on the scaffold or sponge.

Claim 31 (Withdrawn): The bioreactor of claim 30, the hydrostatic loading module being attached to the housing and forming a second chamber with the housing, the second chamber containing a second liquid medium and being separated from the first chamber by the gas permeable membrane.

Claim 32 (Withdrawn): The bioreactor of claim 26, the hydrostatic loading module being attached to the housing and including a second chamber and a third chamber, the second chamber and the third chamber containing a second liquid medium and being separated from the first chamber by, respectively, a first gas permeable membrane and a second gas permeable membrane.

Claim 33(Withdrawn): The bioreactor of claim 26, the plurality of cells being suspended in the first liquid medium.

Claim 34 (Withdrawn): The bioreactor of claim 26, the first liquid medium promoting chondrogenesis.

Claim 35 (Withdrawn): A method of preparing chondrogenic tissue construct, the method comprising:

- isolating a plurality of mesenchymal stem cells from bone marrow;
- expanding the mesenchymal stem cells in a culture medium;
- seeding the expanded mesenchymal stem cells onto a construct;
- growing the seeded construct in a chondrogenic medium; and
- hydrostatically loading the seeded construct while the seeded construct is grown in the chondrogenic medium.

Claim 36 (Withdrawn): The method of claim 35, the seeded construct being grown in the chamber of a bioreactor, the chamber being perfused with the

chondrogenic medium, the bioreactor allowing for hydrostatic loading of the seeded construct in the bioreactor chamber, without removing the seeded construct from the chamber.

Claim 37 (Withdrawn): The method of claim 35, the hydrostatic loading being applied cyclically to the seeded construct.

Claim 38 (Withdrawn): The method of claim 35, the mesenchymal stem cells being treated with a cytokine to promote differentiation to chondrocytes.

Claim 39 (Withdrawn): The method of claim 38, the cytokine comprising fibroblast growth factor 2 (rhFGF-2).

Claim 40 (Withdrawn): The method of claim 35, further comprising,
providing a suspension of mesenchymal stem cells in a culture medium contained in a sterile vessel;
aggregating the mesenchymal stem cells in the vessel,
maintaining the aggregated mesenchymal stem cells in culture for a duration of time sufficient to allow chondrogenesis to begin;
releasing the mesenchymal stem cells from aggregate; and
seeding the construct with the released cells.

Claim 41 (Withdrawn): The method of claim 35, chondrogenic medium containing a first concentration of dexamethasone; and

reducing the concentration of dexamethasone in the chondrogenic medium during growing to a second concentration substantially less than the first concentration, the second concentration of the dexamethasone being effective to induce the expression of BMP-2 in the cells.

Claim 42 (Previously Presented): The bioreactor of claim 1, the at least one gas permeable membrane comprising two membranes having substantially identical gas permeability and being positioned on opposite sides of the first chamber.

Claim 43 (New): The bioreactor of claim 1, the inlet port and the outlet port being sealed during hydrostatic loading to allow the hydrostatic pressure of the first chamber to be increased without loss of first liquid medium from the first chamber.

Claim 44 (New): The bioreactor of claim 3, the second chamber being sealed to pressurize the second liquid medium and thereby transmit hydrostatic pressure through the gas permeable membrane and into the first chamber.